Effect of Levamisole on the Biological Activity of Labeled Iodo-deoxyuridine in EAC Bearing Mice

I. T. Ibrahim[1], K. M.Attallah[1], M.T. El-Kolaly[1], Samy A. Abedelazim[2]

[1]Labeled Compounds Dept. Hot Labs Center, Atomic Energy Authority, [2]Cairo University, Faculty of Pharmacy, Biochemistry Department, Cairo-Egypt.

ismailtaha_73@yahoo.com

***Abstract*-Deoxyuridine (UDR) is a potent antiviral drug could be labeled with radioiodine. ^{125}I-UDR was prepared by electrophilic substitution using iodogen as an oxidizing agent which produce yield above 90%, at pH 7 with heating on a water bath to 75 ^{o}C within 10-15 minute as a reaction time. The labeled drug was found to be stable for 48 h. The labeled compound was investigated using paper electrophoresis and thin layer chromatography. Bio-distribution study of ^{125}I-UDR in ascites bearing mice reflects great uptake of radioactivity in tumor sites. Ehrlish ascites carcinoma cells was injected in the peritoneal cavity of female albino mice to produce ascites. The uptake of ^{125}I-UDR in ascites was increased by combined use of levamisole (LMS) especially at 12h post injection. Mice received LMS showed significant increase in uptake of ^{125}IUDR in tumor site and a significant decrease in other normal sites. LMS also produce significant increase in lymphocytes in normal and tumor bearing mice. Significant increase in hemoglobin, RBCs and WBCs were found in tumor bearing mice received LMS/^{125}I-UDR when compared to control tumor bearing mice. The results of this study encourage the possibility of use ^{125}I-UDR for tumor imaging and therapy.**

***Key Words*-Deoxyuridine; Biodistribution; Levamisole; Ascites/ Lymphocytes.**

I. INTRODUCTION

Radiolabeled nucleosides with the Auger-electron emitting ^{123}I or ^{125}I, have been shown to produce extensive DNA damage in mammalian cell systems *in vitro* [1]. Such nucleosides are cycle-dependent agents that are taken up by mitotically dividing cells in the S phase of the cell cycle [2].The degree of damage that occurs is related to the fact that these nucleosides bind covalently to DNA bringing the decaying Auger electron-emitting radionuclide in close proximity to the genome [3]. The use of this radiohalogenated nucleosides *in-vivo* is associated with several problems. The first problems related to their extremely short biologic half-life in blood ($T_{1/2}$ of minutes in humans). The second problem involved achieving therapeutic ratios in tumor cells in the face of efficient hepatic dehalogenation. This catabolism results in low tissue utilization of the labeled nucleosides and high background blood levels of the circulating radiolabeled metabolite [4].The third problem concerned with the uptake of these radiopharmaceuticals by actively proliferating normal cell, thus potentially causing toxic side effects. The fourth one shared with other cycle-dependent drugs [5]. The lack of selective tumor uptake can lead to radiosensitisation of adjacent normal tissues and enhanced local radiation toxicity [6].

Many trials were attempted like the introduction of the "4'-thio strategy" as a drug design using a practical radioiodine-labeled nucleoside for proliferation imaging [7], [8]. The 5-^{125}I-iodo-4'-thio-2'-deoxyuridine (^{125}I-ITdU) exhibited a high resistance to the glycosidic bond cleavage reaction provoked by thymidine phosphorylase [8]. Also 5-iodo-2-pyrimidinone-2'-deoxyribose (IPdR) was recently reported to be converted to 5-iodo-2'-deoxyuridine (IUdR) by an aldehyde oxidase, mainly concentrated in liver tissue [9]. Other investigator coupled the 5-iodo-2'-deoxyuridine (IUdR) with methotrexate or thymtage to inhibit the rapid degradation of IUdR [10].

Levamisole (LMS) is a synthetic imidazothiazole derivative, and was originally used as an antihelminthic to treat worm infestations in both humans and animals [11]. Its mechanism of action in treating colon cancer is unclear, although it has been shown to have immune-stimulating properties. It is also used infrequently to treat melanoma, head, and neck cancer [12]. Levamisole (LMS) is synergistic with 5-fluorouracil in the adjuvant therapy of patients with stage III colon cancer [13].

The present study was conducted to label Deoxyuridine by electrophilic substitution with iodogen as oxidizing agent. The labeled drug was evaluated against ascites tumor to study the effect of LMS on biodistribution of ^{125}I- UDR. Also effect of ^{125}I- UDR /LMS on blood cells in normal and ascites were studied.

II. MATERIALS A ND METHODS

A. Materials

Deoxyuridine was purchased from Sigma Chemical Company, USA, Levamisole was supplied as a gift from EL-Khera, Ch. Company, Egypt, Iodine-125 was purchased from Isotope Production Center. Newzland, as a no carrier added dissolved in diluted NaOH, Ehrlich ascites carcinoma (EAC) supplied from National Cancer Institute, Cairo, Egypt and all other reagents were of analytical grades.

B. Labeling Techniques

1) Preparation of Iodogen Coated Tubes:

Iodogen was dissolved in chloroform (1mg / ml). The iodogen solution retransferred to tubes in concentrations 5, 10, 25, 50 100, 200 and 300µg. The iodogen solution was evaporated by subjecting the tubes gently to nitrogen gas [14].

2) Labeling Procedures:

The substrate was dissolved in the suitable solvent and transferred to iodogen coated tubes. About 35-70 kbq of Na I^{125} was added and pH, then adjusted to the appropriate one. The reaction solution was left to the suitable reaction time with gentle heating to the suitable temperature at which maximum labeling yield was obtained [15].

The studied factors that affect the percent of labeling yield are oxidizing agent, iodogen content, substrate-content, reaction temperature, pH of the reaction and reaction time.

3) Stability of 125I-DUR :

This experiment was conducted to determine the stability of ^{125}I-DUR after labeling and the impact of time on these compounds. The yield was measured at different time intervals (1, 4, 12, 24 and 48 h) after labeling.

C. Chromatographic Analysis

Two covenants methods were used to investigate the labeled drug from free iodide.

1) Electrophoresis Conditions:

Electrophoresis was done with EC 3000 p-series 90 programmable power and chamber supply units using cellulose acetate strips (45cm). These stripes were moistened with 0.05 M phosphate buffer pH 7 and then were introduced in the chamber. Samples (5 µl) were applied at a distance of 10 cm from cathode. Standing for 1.5 h and application of 300 voltages were done. Developed strips were dried and cut into 1cm segments, then counted by a well-type NaI scintillation counter. The radiochemical yield is calculated as the ratio of the radioactivity of the labeled product to the total radioactivity [16].

Percent Radiochemical yield of ^{125}I-DUR = Peak activity of ^{125}I-DUR X 100 Totala ctivity

2) Thin Layer Chromatography:

Thin layer chromatography was achieved using mobile phase chloroform: ethanol: ammonia (90:10:0.5) with ascending technique. The RF value was about 0.9 for ^{125}I-DUR, while free iodide resist at the point of spotting [17].

D. Tumor Transplantation in Mice

Ehrlich ascites carcinoma cells as an excellent model for studying the biological behavior of malignant tumors and drugs assumed to produce effect at these sites [18]. A line of Ehrlich ascites carcinoma (EAC) was maintained in female Swiss albino mice through weekly IP transplantation of $2.5x10^6$ tumor cells per mouse. EAC cells were obtained by needle aspiration with aseptic condition. The ascetic fluid was diluted with sterile saline so that 0.1 ml contains $2.5x10^6$ cells counted microscopically using a haemocytometer [19]. 0.2 ml solution was then injected intrapritoneally to produce ascites. The animals were maintained for 10-15 days till the tumor development was apparent as described by Olinescu et al., 1983 [20].

E. Bio-distribution of the Labeled 125I-DUR in Tumor Bearing Mice

1) In Ascites Bearing Mice:

This experiment was carried out using 24 ascites bearing mice. The mice were injected with 0.2 ml (5-10 KBq) ^{125}I-DUR in the tail vein and then divided to 4 groups 6 mice each. The mice were kept in metabolic cages for the recommended times (15 min, 1 h, 12 h or 24 h) after injection of labeled drug. Mice were sacrificed by cervical dislocation at various time intervals. Organs and tissues of interest were removed, weighted and counted for its uptake of activity. The counting tubes contain a standard equivalent to 1percent of the injected dose, were assayed in gamma counter and the results were calculated as percentages of injected dose (I.D) per tissue then calculated per gram tissue or organ [21]. The weights of blood, bone and muscles were assumed to be 7, 10 and 40 percent of the total body weight, respectively. Ascites was withdrawn using 20 cm plastic syringe, collected, weighted and counted [15].

2) In Ascites Tumor Bearing Mice Pretreated with Levamisole:

Like the former experiment, group of mice comprise of 24 ascites bearing animals received levamisole (2.5 mg/kg) 24 h, 12 h and 2 h prior administration of the labeled drug [22]. Then the same procedures were done.

a) Hematological Changes

1) In Normal Mice:

Four groups of 6 normal mice were used. The first group was the control, the second group which received 2.5 mg/kg LMS orally daily, the third one received 140KBq of ^{125}IUDR intravenously twice daily and the fourth group received combination of ^{125}IUDR/LMS. The experiment was conducted for 3 weeks. Blood samples were collected from mice and counted for red blood cell count, hemoglobin, white blood cell count and white Blood cell differential count.

In plastic tubes containing EDTA 250 µL of fresh mouse blood was collected. RBC lysis buffer Hema 3 Stain set (388 mM NH4Cl, 29.7 mM NaHCO3, 25 µM Na2EDTA) were used [23].

Hemoglobin Count: One part of blood was added to nine parts of distilled water and hemoglobin was counted colorimetrically in spectrophotometer at wave length 546 and the product were multiplied by 36.77 [24].

Red cell Count: Blood were diluted to 1/1000 in PBS then 10 µL were added to a hemocytometer and the number of RBCs was counted per large square. The product was multiplied by x 1,000 dilution x 10 large squares/µL to give RBC/µL blood [24].

White Cell Count: Mixture of 10 µL whole blood and 190 µL of lysing reagent (a 1/20 dilution) were mixed and incubated for 1 min then 10 µL of lysed blood were examined by hemocytometer. The number of WBC per large square was calculated by WBC/large square x 20 dilution x 10 large squares/µL to give WBC / µL blood [25].

White Cell Differential Count: Fivety µL blood was stained with Leishman stain. 20 µL of the stained blood was spotted near the frosted end of a glass slide. The drop was smeared out across the slide with the end of a second glass slide to obtain a thin film with a smooth feathered edge and examined under microscope [25].

2) In Ascites Bearing Mice:

Four groups of 6 ascites bearing mice each were used and the same procedures as discussed in normal mice.

III. RESULT AND DISCUSION

It was observed that 100 µg iodogen was the optimum content as an oxidizing agent at which maximum labeling yield was obtained. Also 100 µg Deoxyuridine was the optimum content at which maximum labeling yield was obtained. Heating to 75 ^{o}C for 10 min was required to produce maximum yield. PH 7 was the optimum PH value found to obtain maximum labeling yield. Also ^{125}I- DUR was stable for 48h post labeling (Table 1-5).

A. Chromatographic Techniques

1) Electrophoresis Analysis:

It was observed that ^{125}I- DUR persist near the point of spotting, while free iodide move towards the anode. This result was explained as ^{125}I-Vid was neutral compound so no movement towards anode or cathode. On the other hand free iodide carry negative charge so moved towards the anode. This result was in agreement with electrophoresis data obtained for ^{125}I-deoxyuridine [7].

2) Thin Layer Chromatography:

The R_f value was about 0.9 for ^{125}I- DUR, while free iodide persists at the point of spotting in thin layer chromatography.

B. Factors Affecting Labeling Yield

1) Effect of Iodogen Content:

One hundred µg iodogen was the optimum content as an oxidizing. Below this concentration, the yield was significantly decreased, while above this concentration no significant change in the yield was observed.

TABLE 1

EFFECT of IODOGEN CONTENT on the PERCENT YIELD of ^{125}I-DUR

Iodogen (µg)	% Labeled compound	% Free iodide
25 µg	33.0 ± 0.4	67.0 ± 0.4
50 µg	55.5 ± 0.5*+	45.5 ± 0.5
75 µg	74.7 ± 0.8*+	25.3 ± 0.8
100 µg	95.3 ± 0.048*+	4.7 ± 0.048
200 µg	94.3 ± 0.7*+	5.7 ± 0.6

Values represent the mean ±SEM n=6

*Significantly different from the initial values using student's t-test (P<0.05).

+Significantly different from the previous values using student's t-test (P<0.05

2) Effect of Substrate Content:

It was observed that 100 µg substrate was the optimum content (Table 2).

TABLE 2

EFFECT of DEOXYURIDINE CONTENT on the LABELING YIELD of ^{125}I-DUR

Substrate (µg)	% Labeled compound	% Free iodide
25	88.4 ± 0.31	11.6 ± 0.31
50	89.0 ± 0.36	11.0 ± 0.36
100	95.3 ± 0.40*†	4.7 ± 0.40
200	94.5 ± 0.26*	5.5 ± 0.26

Values represent the mean ± SEM. n = 6

*Significantly different from the initial values using unpaired student's t-test (P<0.05).

†Significantly different from the previous values using unpaired student's t-test (P<0.05).

3) Effect of Reaction Temperature:

Temperature may be critical to obtain the maximum yield. In this study 75 °C is the optimum at which maximum yield was obtained.

TABLE 3

EFFECT of TEMPERATURE on the LABELING YIELD of ^{125}I-DUR

Temperature (°C)	% Labeled compound	% Free iodide
25	79.5 ± 0.8	20.5 ± 0.1
50	85.3 ± 1.2 0*	14.5 ±1.2
75	95.3 ± 0.45*†	4.7 ± 0.45
100	91.1 ± 0.7*†	8.9 ± 0.7

Values represent the mean ± SEM. n = 6

*Significantly different from the initial values using unpaired Student's t-test P<0.05).

†Significantly different from the previous values using unpaired Student's t-test (P<0.05).

4) Effect of pH :

The neutral medium (pH7) using phosphate buffer result in obtaining maximum yield (Table 4).

TABLE 4

EFFECT of pH of the REACTION MEDIUM on the LABELING YIELD of ^{125}I-DUR

pH value	% Labeled compound	% Free iodide
3.0	92.0 ± 0.30*	8.0 ± 0.30
7.0	95.3 ± 0.80*†	4.7 ± 0.30
9.0	87.3 ±1.2*†	12.7 ± 1.2
10.8	25.6 ± 0.44*†	74.4 ± 0.44
11.0	2.5 ± 0.20*†	97.5 ± 0.20

Values represent the mean ±SEM. n = 6

*Significantly different from the initial values using unpaired Student's t- test (P<0.05).

†Significantly different from the previous values using unpaired Student's t-test (P<0.05).

5) Effect of Reaction Time:

The reaction should proceed for 30 min to give the maximum yield. Exceeding or decreasing the reaction time from 30min produce significant decrease in the yield (Table 5).

TABLE 5

EFFECT of REACTION TIME on the PERCENT LABELING YIELD of ^{125}I-UDR

Time (min)	% Labeled compound	% Free iodide
1	36.9 ± 0.36	63.1 ± 0.36
5	44.8 ± 0.49*	55.2 ± 0.49
15	81.3 ± 0.21*†	18.7 ± 0.21
30	95.3 ± 0.35*†	4.7 ± 0.35
60	89.0 ± 0.7 *†	10.0 ± 0.7

Values represent the mean ± SEM. n = 6

*Significantly different from the initial values using unpaired Student's t- test (P<0.05).

†Significantly different from the previous values using unpaired Student's t-test P<0.05).

*6) In-vitro Stability of ^{125}I-Deoxyuridine***:**

The labeled ^{125}I-deoxyuridine was stable for 48 h post labeling Table (6).

TABLE 6

EFFECT of TIME on the STABILITY of ^{125}I-DUR

Time (h)	% Labeled compound	% Free iodides
1	95.3 ± 0.35	4.7 ± 0.35
4	95.3 ± 0.35	4.7 ± 0.35
12	95.2 ± 0.45	4.8 ± 0.45
24	95.1 ± 0.49	4.9 ± 0.49
48	95.1 ± 0.49	4.9 ± 0.49

7) Bio-distribution of ^{125}I-UDR

a) In ascites bearing mice:

Table (7) represented the data of bio-distribution of ^{125}I-UDR in ascites bearing mice. ^{125}I-UDR rapidly distributed in most of tissues. Blood, stomach, lung, heart and kideny were the organs of highest ^{125}I-UDR uptake at 15 minute post injection. Bone, muscle, liver, ascites, stomach and thyroid were organs which produced significant increase in ^{125}I-UDR uptake at 1h post injection. At the other hand, blood, lung, heart and kideny showed significant decrease in ^{125}I-UDR uptake at the same time post injection. Significant increase in ^{125}I-UDR uptake was observed only in Ascites and thyriod at 12h post injection, while others produce significant decrease in ^{125}I-UDR uptake. The majority of tissues produce significant decrease in ^{125}I-UDR uptake at 24 h post injection**.**

TABLE 7

BIODISTRIBUTION of ^{125}I-UDR in ASCITES BEARING MICE

Organs & body fluids	Percent I.D./gram organ, Time post injection			
	15 min	1 h	12 h	24 h
Blood	22.5 ± 1.10	13.1 ± 0.7*	6.6 ± 0.1*	1.50 ± 0.03*
Bone	2.40 ± 0.05	3.5 ± 0.1*	1.5 ± 0.15*	0.50 ± 0.01*
Muscle	0.5 ± 0.09	1.8 ± 0.01*	0.9 ± 0.01*	0.50 ± 0.02*
Liver	2.0 ± 0.25	3.4 ± 0.2*	2.4 ± 0.01*	0.60 ± 0.01
Lung	8.0 ± 0.10	6.0 ± 0.2*	2.0 ± 0.2*	0.75 ± 0.01*
Heart	7.0 ± 0.30	5.0 ± 0.40*	2.0 ± 0.01*	0.50 ± 0.1*
Stomach	12.0 ± 0.90	13.2 ± 0.9*	7.7 ± 0.4*	3.60 ± 0.2*
Intestine	5.1 ± 0.060	4.4 ± 0.05*	3.4 ± 0.05*	1.80 ± 0.03
Kidney	4.6 ± 0.40	3.3 ± 0.1*	2.1 ± 0.05*	1.70 ± 0.1*
Spleen	1.6 ± 0.10	3.1 ± 0.1*	1.5 ± 0.01*	0.70 ± 0.01*
Thyroid	1.0 ± 0.02	4.0 ± 0.3*	12.5 ± 0.3*	12.40 ± 0.2
Ascetic fluid	2.1 ± 0.20	4.4 ± 0.25*	5.5 ± 0.19*	1.50 ± 0.14*

Values represent mean ± SEM. n = 6

*Significantly different from each previous value of each organ using unpaired Student's t-test (P<0.05).

b) In Ascites Bearing Mice Pretreated with Levamisole:

The uptake of ^{125}I-UDR was observed in most of organs e.g. blood, heart, stomach and thyroid. While ^{125}I- UDR uptake was significantly increased in ascites pretreated with levamisole that was observed at 1, 12, 24h post injection. ^{125}I-UDR uptake in blood decline rapidly and show significant decrease at all time intervals. At 15 minutes post injection, blood, heart, kidney and stomach were the organs of highest ^{125}I-UDR uptake. While muscle, thyroid and spleen were the organs of lowest uptake. At 1h post injection, the majority of organs showed significant decrease in ^{125}I-UDR uptake. The same observation was noticed at 12h post injection except that stomach which showed significant decreases in ^{125}I-UDR uptake. Ascites showed no significant change in ^{125}I- UDR uptake at 24h post injection while the majority of organs showed significant decrease (Table 8). Only the thyroid showed significant increase in ^{125}I- UDR uptake at 24 h post injection, which may be due to in-vivo deiodination of ^{125}I-UDR [26].

These results when compared with that of biodistribution of ^{125}I-UDR in ascites bearing mice, it was observed that some organs showed significant decrease in ^{125}I-UDR uptake like blood, heart and stomach. At the other hand, significant increase in ^{125}I-UDR uptake was observed in ascites.

TABLE 8

BIODISTRIBUTION of ^{125}I-UDR in ASCITES BEARING MICE PRETREATED with LEVAMISOLE

Organs & bodyfluids	Percent I.D./gram organ Time post injection			
	15 min	1 h	12 h	24 h
Blood	21.9 ± 1.3	12.1 ± 0.7*	6.3 ± 0.1*	1.30 ± 0.03*
Bone	2.1 ± 0.05	3.2 ± 0.1*	1.3 ± 0.1*	0.40 ± 0.01*
Muscle	0.5 ± 0.09	1.7 ± 0.01*	0.8 ± 0.01*	0.40 ± 0.02*
Liver	2.0 ± 0.25	3.4 ± 0.2*	2.4 ± 0.01*	0.50 ± 0.01
Lung	7.6 ± 0.10	5.6 ± 0.2*	1.8 ± 0.1*	0.75 ± 0.01*
Heart	6.7 ± 0.30	4.7 ± 0.40*	2.0 ± 0.01*	0.50 ± 0.1*
Stomach	11.5± 0.90	12.2± 0.9*	7.4 ± 0.4*	3.20 ± 0.2*
Intestine	5.0 ± 0.06	4.0 ± 0.05*	3.2 ± 0.05*	1.70 ± 0.03
Kidney	4.3 ± 0.40	3.0 ± 0.1*	2.0 ± 0.05*	1.60 ± 0.1*
Spleen	1.6 ± 0.10	3.1 ± 0.1*	1.5 ± 0.01*	0.70 ± 0.01*
Thyroid	1.0 ± 0.02	3.7 ± 0.3*	11.5± 0.3*	14.40 ± 0.2*
Ascitic fluid	2.5 ± 0.20	4.6 ± 0.25*	6.0 ± 0.19*	2.50 ± 0.14*

Values represent mean ± SEM. n = 6

*Significantly different from each previous value of each organ using unpaired Student's t-test (P<0.05).

8) Hematological Changes

a) In Normal Mice:

1. Effect on the Hemoglobin Level:

It was observed that at 10, 20 and 30th days post administration of drugs all groups showed non significant difference when compared to control group as the mean was 12.7 ± 0.502. Group received ^{125}I-UDR/LMS showed the same result like group received LMS.

2. Effect of the Drugs on RBCs Count:

As the previous results, the same was observed for hemoglobin level as all groups showed non significant difference when compared to control group as the mean 8.25 ±

0.24. Group received ^{125}I-UDR/LMS showed the same result like group received LMS.

3. Effect of the Drugs on WBCs Count:

Significant increase in the count of WBCs was observed in levamisole received group at 20 and 30th days post administration. On the other hand, group received ^{125}I-UDR showed no significant change when compared to control group as in Table (9). Group received ^{125}I-UDR/LMS showed the same result like group received LMS.

TABLE 9

EFFECT of DRUGS on WBCs COUNT

Animal groups	10th day	20th day	30th day
Control	7628.1 ± 78.50	7688.8 ± 98.50	7705.5 ± 105.65
^{125}I-UDR	7618.5 ± 82.4	7680.7 ± 95.45	7770.5 ± 110.6
LMS	7780.8 ± 95.52	7980.9 ± 100.7^{+}	8009.5 ± 115.75^{+}

Values represent the mean ±SEM n=6

*Significantly different from the values of control using student's t- test (P<0.05).

+Significantly different from the previous values in the same raw using student's t- test (P<0.05)

4. Effect of the Drugs on Neutrophils Count:

The count of neutrophils showed significant increase in levamisole received group at 20 and 30th day post administration, while ^{125}IUDR showed no significant change when compared to control group as in Table (10). Group received ^{125}I-UDR/LMS showed the same result like group received LMS.

TABLE 10

EFFECT of DRUGS on NEUTROPHILS (COUNT/CU MM)

Animal groups	10th day	20th day	30th day
Control	1430.1 ± 14.50	1490.18 ± 15.3	1495.25 ±14.6
^{125}I-UDR	1427.5 ± 15.4	1476.70 ± 21.5	1485.50 ± 21.3
LMS	1447.8 ± 15.2	1520.90 ±21.7†	1558.90 ± 19.5†

Values represent the mean ±SEM n=6

†Significantly different from the previous values in the same column using student's t- test (P<0.05)

*Significantly different from the values of control using student's t- test (P<0.05).

5. Effect of Drugs on Lymphocytes (count /cu mm):

Significant increase in the count of lymphocytes was observed in levamisole received group at 20 and 30 days post administration No significant change was observed in group received ^{125}I-UDR when compared to control group as in (Table 11). This may be due to the immunostimulatory effect of LMS [13]. Group received ^{125}I-UDR/LMS showed the same result like group received LMS.

TABLE 11

EFFECT of the DRUGS on LYMPHOCYTES (COUNT/CU MM)

Animal groups	10th day	20th day	30th day
Control	5762.1 ± 17.5	5688.8 ± 18.5	5905.5 ± 11.65
^{125}I-UDR	5718.5 ± 14.4	5680.7 ± 19.5	5850.5 ± 11.6
LMS	5880.8 ± 15.52 †	5980.9 ± 12.7 † #	6300.5 ± 19.75 † #

Values represent the mean ±SEM n=6

*Significantly different from the values of control using student's t- test (P<0.05).

†Significantly different from the previous values in the same raw using student's t- test (P<0.05).

b) In Ascites Tumor Bearing Mice

1. Effect on the Hemoglobin Level:

At 5th day no significant change in hemoglobin level was observed in all groups when compared to the control group. At 15th day post inoculations all groups showed significant decrease in hemoglobin level when compared to data at 10 days post inoculation. At the same time significant increase in hemoglobin level was observed in ^{125}I-UDR /LMS and ^{125}I-UDR received groups when compared to control group. At 20th days post inoculation, significant increase in hemoglobin was observed in ^{125}I- UDR and levmisole received groups (Table12). The explanation of these results may be due to the antitumor effect of ^{125}I-UDR which may be also enhanced by immunostimulatory effect of LMS [27].

TABLE 12

EFFECT of the DRUGS on HEMOGLOBIN LEVELS (g/dl) in ASCITES BEARING MICE

Animal groups	5th day	10th day	15th day	20th day
Control	10.80 ± 0.24	10.3 ± 0.15	8.80 ± 0.25	7.30 ± 0.44
Levamisole	10.80 ± 0.32	10.4 ± 0.19	9.10 ± 0.15	8.75 ± 0.17*†
^{125}I-UDR	11.15 ± 0.4	10.7 ± 0.5*	10.10 ± 0.18*†	10.20 ± 0.14*†
^{125}I-UDR/LMS	11.10 ± 0.3	10.8 ± 0.55*	10.25 ± 0.18*	10.70 ± 0.4*

†Significantly different from the previous values in the same column using student's t- test (P<0.05

*Significantly different from the values of control using student's t- test (P<0.05).

2. Effect on RBCs Count in Tumor Bearing Mice:

After 5 days post inoculation, except for ^{125}I-UDR/LMS and ^{125}I-UDR received group, the other groups showed significant decrease in RBC count when compared to the value of the same group at 5th day post inoculation While LMS, ^{125}I-UDR and ^{125}I-UDR/LMS received groups produced significant change when compared with control. At 15th day post inoculation all groups showed significant decrease in RBC count when compared to its corresponding values at 10th day post inoculation. ^{125}I-UDR and ^{125}I-UDR/LMS received groups showed significant increase in RBC when compared to value of the control at the same day. The same result was observed at 20th day post inoculation (Table 13).

TABLE 13

EFFECT of the DRUGS on RBCs COUNT (x10 ^{6}/cu mm) in TUMOR BEARING MICE

Animal groups	5th day	10th day	15th day	20th day
Control	6.10 ± 0.2	4.3 ± 0.1	3.8 ± 0.25	3.30 ± 0.4

Levamisole	6.20 ± 0.3	5.4 ± 0.19*	4.1 ± 0.15	3.75 ± 0.17*
^{125}I-UDR	6.15 ± 0.3	5.7 ± 0.35*	4.6 ± 0.1*†	4.80 ± 0.14*†
^{125}I-UDR/LMS	6.10 ± 0.2	5.8 ± 0.5*	4.8 ± 0.2 8*	4.70 ± 0.4*

† Significantly different from the previous values in the same column using student's t- test (P<0.05

*Significantly different from the values of control using student's t- test (P<0.05).

3. Effect on WBCs Count in Tumor Bearing Mice:

It was observed that WBCs highly affected by LMS and when coupled ^{125}I-UDR. Levamisole decreases the total count of WBCs whither alone or coupled with ^{125}I-UDR when compared to control tumor bearing group (Table 14). To explain this result we should take in consideration that tumor itself cause increase in the number of WBCs which stimulated by the immune system to fight tumor cells. The WBCs in group received ^{125}I-UDR was little than in control tumor mice because of the antitumor effect of ^{125}I-UDR which may be enhanced by LMS [12].

TABLE 14

EFFECT of the DRUGS on WBCs (COUNT/CU MM) in ASCITES BEARING MICE

Animal groups	**5th day**	**10th days**	**15th day**	**20th day**
Control	17492.1 ± 78.50	17443.0 ± 61	18883 ± 85	14443 ± 44
Levamisole	16498.5 ± 80.4*	14654.0 ± 19*	11451 ± 55*	9653 ± 17*
^{125}I-UDR	16975.5 ± 65.5*	13665.7 ± 35*	10300 ± 56*	8888 ± 0.14*
^{125}I-UDR/LMS	14962.1 ± 88.50*	10855.8 ± 65*	9455 ± 88*	7986 ± 0.4*

† Significantly different from the previous values in the same column using student's t- test (P<0.05

*Significantly different from the values of control using student's t- test (P<0.05).

4. Effect of the Drugs on Percent Lymphocytes in Tumor Bearing Mice:

At 5th day post inoculation only LMS received group showed significant increase in lymphocytes count while non significant change showed in the others. Significant decrease in lymphocyte count was observed in all groups at 10th day post injection when compared to its correspondence value at 5th day post inoculation. Significant increase was observed in ^{125}I-UDR and ^{125}I-UDR/LMS received groups at 10th day post inoculation when compared to control. Significant decrease was observed in ^{125}I- UDR/LMS and ^{125}I-UDR received groups when compared to data at 15th day post inoculation (Table15).

TABLE 15

EFFECT of DRUGS on PERCENT LYMPHOCYTES (COUNT/CU MM) in TUMOR BEARING MICE

Animal groups	**5th day**	**10th day**	**15th day**	**20th day**
Control	45 ± 1.5	33 ± 0.75	25 ± 0.95	21 ± 0.7
Levamisole	51 ± 0.5*†	41 ± 1.25*	35 ± 1.5*	28 ± 0.45*
^{125}I-UDR	45 ± 2.5†	36 ± 2.5*†	38 ± 0.65	45 ± 0.85*†
^{125}I-UDR/LMS	44 ± 1.75	39 ± 0.8	46 ± 0.5*†	56 ± 0.65*†

†Significantly different from the previous values in the same column using student's t- test (P<0.05)

*Significantly different from the values of control using student's t- test (P<0.05).

5. Effect of the Drugs on Percent Neutrophils (count /cu mm) in Ascites Bearing Mice:

Control group showed significant increase in percent neutrophils at 10, 15, and 20th days post inoculation when compared to the value at 5th day post inoculation. At 5th day post inoculation all groups showed no significant change in percent neutrophils except those received LMS when compared to the control group. Significant change in percent neutrophils in all groups were observed when compared to the percent neutrophils at 5th day post inoculation. Only ^{125}I-UDR received group showed non significant change in percent neutrophils. At 20th day post inoculation all groups showed significant change in percent neutrophils (Table16).

TABLE 16

EFFECT of DRUGS on PERCENT NEUTROPHILS (count /cu mm) in ASCITES BEARING MICE

Animal groups	**5th day**	**10th day**	**15th day**	**20th day**
Control	53.0 ± 1.5	65.0 ± 0.75	73.0 ± 0.95	78.0 ± 0.7
Levamisole	48.7 ± 0.5*	57.1 ± 1.25*	63.5 ± 1.5*	70.0 ± 0.45*
^{125}I-UDR	53.4 ± 2.5†	62.0 ± 2.5*†	60.2 ± 0.65*	53.5 ± 0.85*†
^{125}I-UDR/LMS	54.8 ± 1.75	60.0 ± 0.8*	64.0 ± 0.5*†	42.3 ± 0.65*†

† Significantly different from the previous values in the same column using student's t- test (P<0.05

*Significantly different from the values of control using student's t- test (P<0.05).

IV. CONCLUSION

^{125}IUDR was prepared by electrophilic substitution using iodogen as with yield above ninety percent, at pH 7. The labeled drug was stable for 48h. Biodistribution study of ^{125}I-UDR in ascites bearing mice reflects great uptake of radioactivity in tumor sites. LMS increase uptake of ^{125}I-UDR in tumor sites and produce significant changes in hematological parameters. These results encourage the use of immunomodulators with other agents to fight tumors.

REFERENCES

[1] J.G. Tujvajev, H.A. Macapinlac and F. Daghighian, J. Nucl. Med., 35, 1407, 1994.

[2] S.J. Adelstein and A.I. Kassis, Acta Oncol., 35 (7), 797, 1996.

[3] A.I. Kassis, A.M. Kirichian, K. Wang, E.S. Semnani and S.J. Adelstein, Int. J. Radiat. Biol., 80, 941, 2004.

[4] A.I. Kassis, J. Nucl. Med., 44, 1479, 2003.

[5] J. Pressacco, D.W. Hedley and C. Erlichman, Cancer Res., 54, 3772, 1994.

[6] H.F. Beer, S. Lin, I. Novak-Hofer and P.A. Schubiger, Appl. Radiat. Isotopes, 46, 781, 1992.

[7] J. Mester, K. DeGoeij and M. Sluyser, Eur. J. Cancer, 32, 1603, 1996.

[8] I Kassis, "Molecular and cellular radiobiological effects of Auger emitting radionuclides," Radiat. Prot. Dosimetry, 143 (2-4), 241, 2011.
L. Bodei, A.I. Kassis, S.J. Adelstein and G. Mariani, Cancer Biother. Radiopharm., 18 (6), 861, 2003.

[9] Catherine, S. Foulon and A. James, J. Nucl. Med., 37, 1S, 1996.

[10] C.C. Hook, D.W. Kimmel and L.K. Kvols, Ann. Neurol., 31, 262, 1992.

[11] R.S. Mutch and P.R. Hutson, Clin. Pharm., 10, 95, 1991.

[12] Yuan, R. Bibi and T. Dang, Int. Immunol., 16 (5), 707, 2004.

[13] K. Chen, S.J. Adelstein and A.I. Kassis, Int. J. Radiat. Biol., 80 (11-12), 921, 2004.

[14] H.H. Coenen and D.S. Wilbur, Bioconjugate chem., 3, 433, 1992.

[15] J.W. Darnowski and F.A. Goulette, Biochem. Pharmacol., 48,1797, 1994.

[16] I. Kassis, "Therapeutic radionuclides: biophysical and radiobiologic principles," Semin. Nucl. Med., 38 (5), 358, 2008.

[17] M.A.Walicka, Y. Ding, A.M. Roy , R.S. Harapanhalli, S.J. Adelstein and A.I. Kassis, Int. J. Radiat. Biol., 75 (12), 1579, 1999.

[18] Korde, M. Venkatesh and H.D. Sarma; "Int. Symposium on Modern

[19] Trends in Radiopharmaceuticals for Diagnosis and Therapy", Lisbon, Portugal, 30 March-3 April, IAEA, SM-355/13 (1998).

[20] Olinescu, S. Hristescu and E. Mazilu, Neoplasma, 30 (2), 147, 1983.

[21] A.I. Kassis, "Radiotargeting agents for cancer therapy," Expert Opin Drug Deliv, 2 (6), 981, 2005.
H.C. Hogland, Semin Oncology, 9, 95, 1982.

[22] S.B. Prasad and A. Giri, Indian J. Expt. Biol., 32, 155, 1994.

[23] A.K. Abbas and A.H. Lichtman, Cellular and Molecular Immunology, 5th ed., Saunders, Philadelphia, USA, 2003.

[24] A.I. Kassis and S.J. Adelstein, J. Nucl. Med., 46, 4S, 2005.

[25] P. Balagurumoorthy, K.Chen, S. J. Adelstein and A. I. Kassis, "Auger electron-induced double-strand breaks depend on DNA topology," Radiat. Res.,

[26] 170 (1), 70, 2008.

[27] K. Wang, S. J. Adelstein, A. I. Kassis, "DMSO increases radioiodination yield of radiopharmaceuticals," Appl. Radiat. Isotopes, 66 (1), 50, 2008.